International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 8 | Issue 2 | APR-JUN | 2018 | 579-582
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

DETECTION OF GROUNDNUT BUD NECROSIS VIRUS IN GROUNDNUT BY DAC-ELISA IN ANDHRA PRADESH, INDIA

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ABSTRACT

Groundnut bud necrosis virus (GBNV) or peanut bud necrosis virus (PBNV) causes peanut bud necrosis disease (PBND) in groundnut belongs to the family Bunyaviridae, the genus Tospovirus. PBND was first reported in India and later from South and Southeast Asia. GBNV is mainly transmitted by Thrips and account for immense yield loss up to 90 percent in groundnut. The hot-spot areas for PBND are Jagtiyal, Hyderabad and Kadiri in Telangana and Andhra Pradesh (AP), respectively. Prevention and control of a disease depends on the early and accurate detection of pathogen. ELISA was proven to be a best diagnostic method to detect plant viruses accurately. Hence, in this study, we used DAC-ELISA as a diagnostic tool to detect GBNV in groundnut. The symptoms like chlorotic spots and necrotic rings on leaves, stunted growth and wilting were noticed on some of the groundnut plants in groundnut fields of Surathkhan palli and Sreenivasapuram villages of Kadapa district, AP. We initially suspected for PBND and the symptomatic plant leaf materials (31 leaf samples) were randomly collected and subjected for DAC-ELISA. The GBNV antisera positively reacted with two groundnut sample of Sreenivasapuram by DAC-ELISA.

KEY WORDS

Direct antigen coating (DAC)-ELISA, Groundnut bud necrosis virus, Groundnut, Peanut bud necrosis disease.

INTRODUCTION

Groundnut or peanut (*Arachis hypogaea* L.) is an annual oilseed, food and fodder crop grown in diverse environments all over the world between 40^0N and 40^0S [1]. According to FAOSTAT (2016), world-wide groundnut harvested area and production were 27.7 million ha with 44 million tonnes in which India 5.8 million ha and 6.8 million tonnes, respectively. India ranked second in production with 5 million tonnes from an area of 5 million ha next to China [2]. In India, the major cultivating states were Maharashtra, Gujarat, Andhra Pradesh, Karnataka, Tamil Nadu and Rajasthan contributes about 90 percent of the total groundnut production. The groundnut is susceptible to many diseases caused by fungi, viruses, bacteria and nematodes. Among these pathogens viruses have great economic impact on groundnut production. Nearly around 31 viruses representing 14 groups were reported to naturally infects groundnut worldwide [1]. Of these groundnut viruses, "GBNV, *tobacco streak virus* (TSV), *peanut mottle virus* (PeMoV), and *Indian peanut clump virus* (IPCV)" are economically important in India [3]. GBND in groundnut was first reported from India and named as "bud necrosis" by Reddy *et al.,* [4]. This disease was coined under many alternative names as groundnut mosaic, chlorosis, ring mottle, ring spot, groundnut rosette, bunchy top, bud blight and ring mosaic. Under field conditions, GBNV also infects several other crops including mungbean, soybean, urdbean, cowpea, sunflower, pea, carrot, lablab, tomato and cotton [5]. The hot-spot locations for PBND incidence in India are Kadiri, Rajendra nagar and Palem (Andhra Pradesh), Mainpuri (Uttar Pradesh), Latur (Maharashtra), Raichur (Karnataka) and Tikamgarh (Madhya Pradesh) [6]. The rate of disease incidence depends on the time of infection and stage of crop

growth in groundnut. In India, PBND incidences account for 30 to 90 percent yield loss in groundnut [7,6]. The characteristic symptoms of GBNV in infected groundnut plants are chlorotic and necrotic rings, stunting and proliferation of axillary shoots, distortion of lamina, mosaic, mottling, chlorosis, decrease in size, rarely shoestring appearance of leaflets, and bushy appearance of the plant [3]. The virus is naturally and experimentally transmitted by *Thrips palmi* and sap inoculation, respectively. GBNV is a single stranded, negative sense membrane-bound RNA virus belongs to the family *Bunyaviridae*, genus *Tospovirus*. Of the four serogroups of *Tospovirus*, GBNV belongs to the serogroup IV [8]. In the present study, we have detected the bud necrosis disease in the groundnut field samples by using GBNV polyclonal antibodies in DAC-ELISA.

MATERIALS AND METHODS

Sample collection

During the field inspection, the leaf samples of the groundnut showing chlorotic spots and necrotic rings on leaves, stunted growth and wilting were randomly collected from Surathkhan palli and Sreenivasapuram of Kadapa district, AP. A total of 31 leaf samples i.e. 15 samples from Surathkhan palli and 16 samples from Sreenivasapuram were collected and subjected to DAC-ELISA for the detection of GBNV.

DAC-ELISA

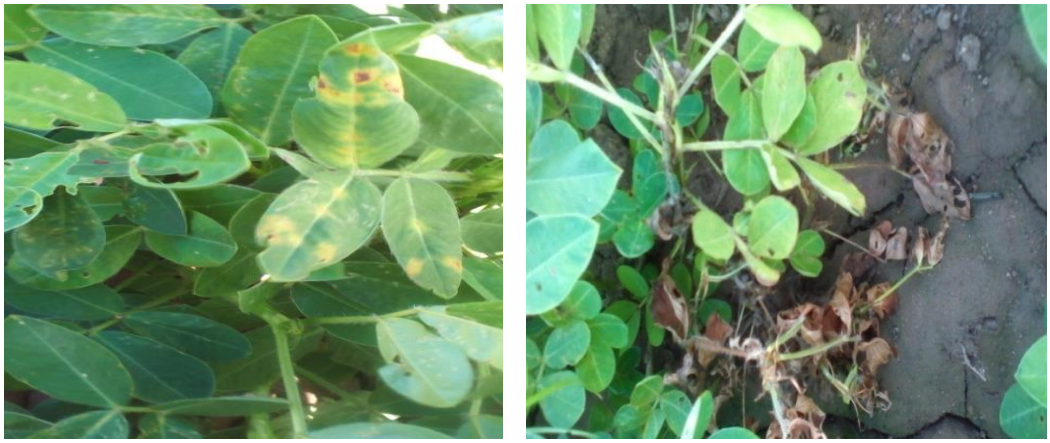
DAC-ELISA was carried out as described by Clark and Joseph [9]. The leaf samples suspected for PBND washed with sterile distilled water and macerated in a carbonate buffer (0.2 M $NaHCO_3$; 0.2 M Na_2CO_3; pH 9.6) to a concentration of 1:10 (w/v). A healthy control was maintained. The leaf extracts of 200 µl were dispensed to the wells of the ELISA microplate and incubated for 2 hr at 37^0C.Then the wells were washed with PBS-T (0.1 M PBS with 0.1% Tween 20) and PBS buffers (pH 7.4). To the wells 200 µl of GBNV antiserum diluted (1: 5000, v/v) in antibody buffer (PBS-T; 2% PVP; 0.2% egg albumin) was dispensed into the wells and incubated at 37^0C for 90 min. After completion of incubation period, the plate was washed with PBS-T and PBS buffers. Alkaline phosphatase (ALP) labelled secondary antibodies at 1:2000 dilution (v/v) were loaded (200µl) into the wells and again incubated for 90 min at 37^0C. Then the plate was washed with PBS-T and PBS buffers and added 200 µl of substrate solution (Diethanolamine, 9.7 ml; Distilled water, 80 ml; p-Nitrophenyl phosphate 0.5 mg/ml; pH 9.8) and incubated in dark place for 30 min at room temperature. The reaction was arrested by adding 50 µl of 2 N NaOH solution and OD values taken at A_{405nm} in an ELISA plate reader (BIORAD).

RESULTS AND DISCUSSION

Groundnut (*Arachis hypogaea* L) is the largest oil producing crop in India known as the "Kings of oil seeds" [10]. The disease "bud necrosis" was reported to be the major constraint to the groundnut production in South and Southeast Asia. The pathogen for this disease is *Groundnut bud necrosis virus* (GBNV) reported first from India in 1968 [11]. The other viruses of groundnut in India are " *peanut chlorotic leaf streak virus* (PCLSV), *tomato spotted wilt virus* (TSWV), *peanut mottle virus* (PeMoV), *Indian peanut clump virus* (IPCV), *tobacco streak virus* (TSV), *peanut stripe virus* (PStV), *groundnut yellow mosaic virus* (GYMV), *peanut green mosaic virus* (PGMV), *peanut crinkle virus, groundnut ring spot, groundnut rosette assistor virus* (GRAV), *peanut yellow spot virus* (PYSV) and *groundnut rosette virus* (GRV, satellite RNA)" [1]. In addition to groundnut, GBNV also infects soybean, tomato, potato and mung bean [12, 13 & 14]. In the present study we have visited groundnut fields of villages of Sreenivasapuram and Surathkhan palli of Kadapa district, AP. In the fields, some of the plants showing chlorotic spots and necrotic rings on leaves, stunted growth and wilting (Fig.1). The symptoms like faint chlorotic spots and mottling were observed on young leaflets and later these symptoms turned into chlorotic and necrotic rings on groundnut plants [3]. Based on the symptoms we have suspected the plants were infected by bud necrosis disease. However, other pathogens of groundnut also show similar type of symptoms on plants. Therefore, to know the pathogen of the disease we have randomly collected 31 symptomatic leaf samples (15 samples Surathkhan palli and 16 samples from Sreenivasapuram) and tested for GBNV by DAC-ELISA. The OD values of the two positively reacted samples were thrice than the healthy groundnut sample. The OD value of the test sample is more than two folds than the OD values of the control sample considered as test positive [16]. The polyclonal antibodies of GBNV were reacted positively with two samples of Sreenivasapuram. All the samples of Surathkhan palli were negative for GBNV indicating that the pathogen(s) other than the GBNV was involved and

expressed such kind of symptoms on groundnut plants. Various formats PCR techniques can be used to detect the GBNV but these were expensive and not compatible to field application. ELISA is a less cumbersome, sensitive (based on the quality of antisera) and field compatible to detect the virus in plants.

Fig.1. The infected groundnut leaf showing chlorotic and necrotic spots.



CONCLUSION

Groundnut plants showing chlorotic spots and necrotic rings on leaves, stunted growth and wilting were tested for bud necrosis disease by DAC-ELISA and detected GBNV in two samples of Sreenivasapuram villages of Kadapa district, Andhra Pradesh. The remaining symptomatic plants may be infected by other than the GBNV.

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